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	SampleName	Inj. Volume	Channel	Dilution
1	K63 In PBS	100,00	214nm	4,00
2	K63 In Chaps 0,25%	100,00	214nm	4,00
3	K63 In citrate	100,00	214nm	4,00

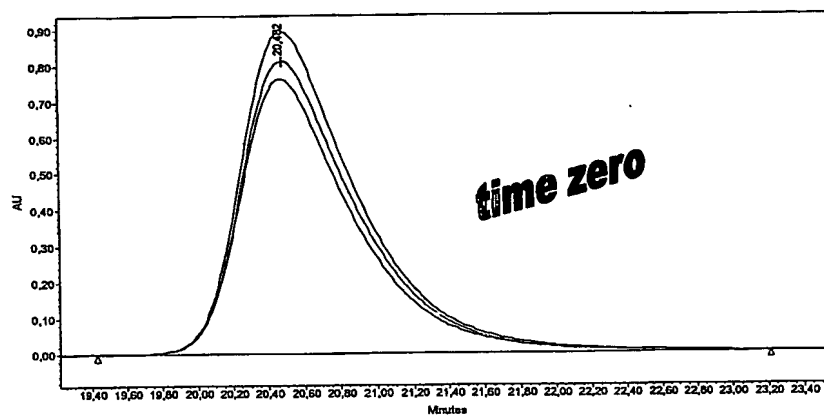


Figure 1A

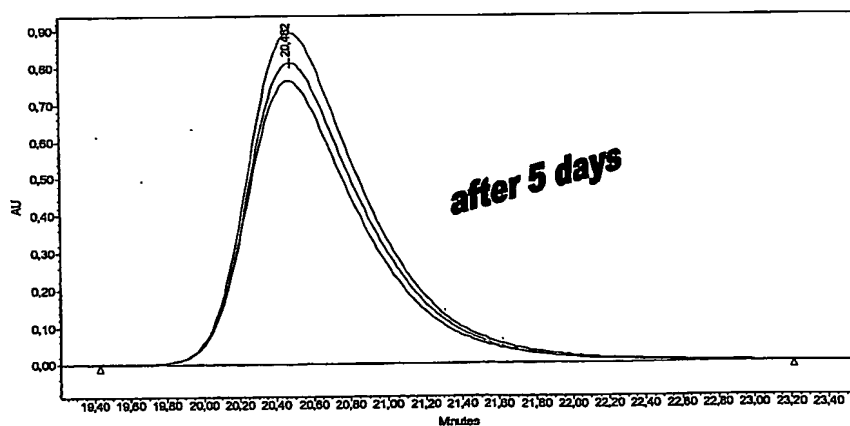
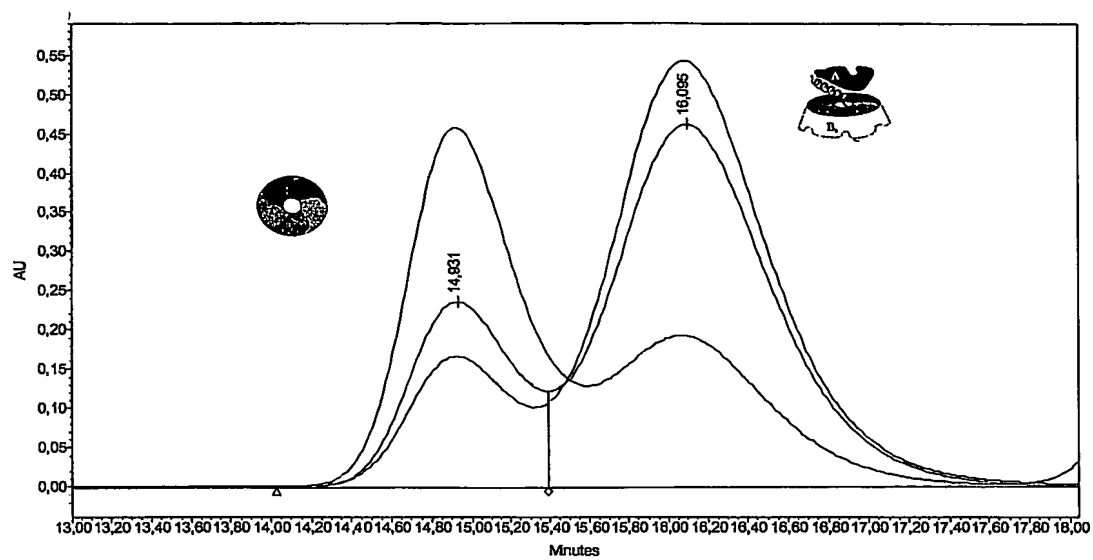


Figure 1B

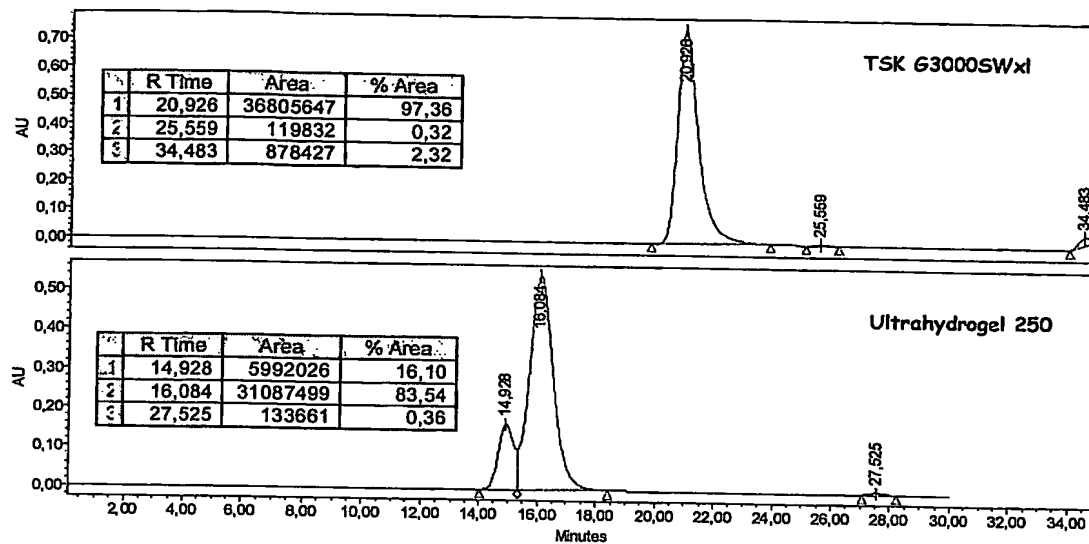
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Figure 1C



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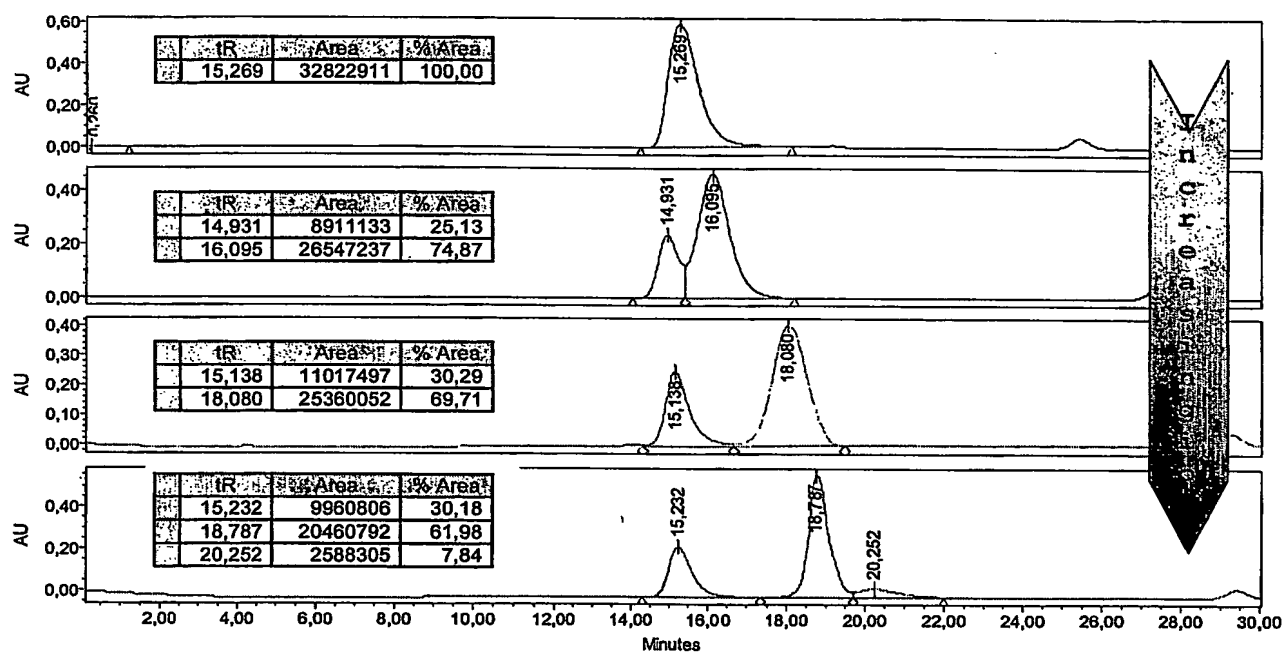
Figure 1D



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Figures 2A-2D

	Sample Name	Date Acquired	Eluent	Injection Volume	Channel	Dilution
1	PBS 5gg agitazione	09/04/2003 9.55.19	KPI 50 mM + Na2SO4 50 mM pH 7,2	100,00	214nm	4,00
2	PBS 5gg agitazione	08/04/2003 13.53.08	KPI 100 mM + Na2SO4 100 mM pH 7,2	100,00	214nm	4,00
3	PBS 5gg agitazione	09/04/2003 15.07.11	KPI 250 mM + Na2SO4 100 mM pH 7,2	100,00	214nm	4,00
4	PBS 5gg agitazione	10/04/2003 9.51.42	KPI 200 mM + Na2SO4 200 mM pH 7,2	100,00	214nm	4,00



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Figure 3

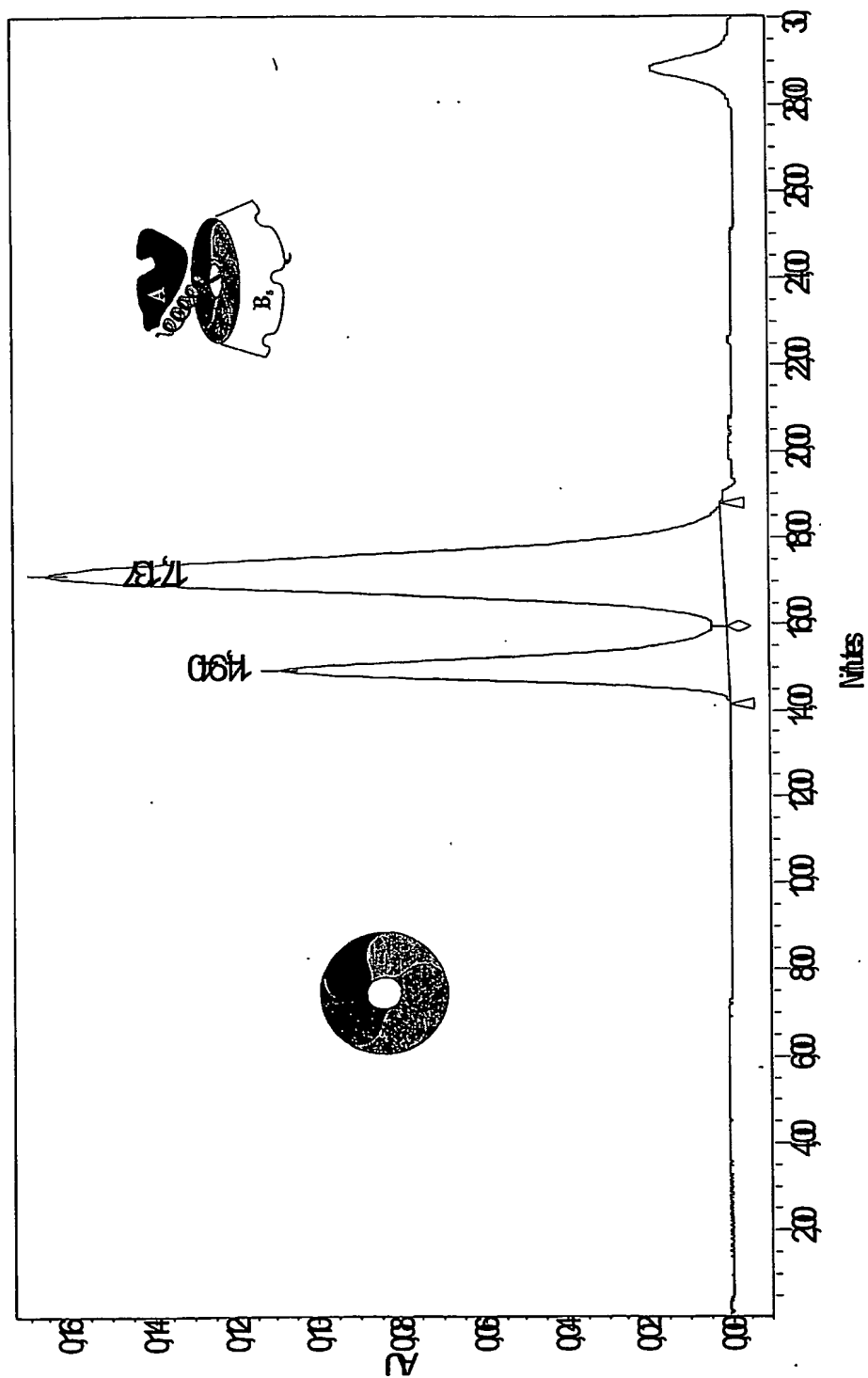
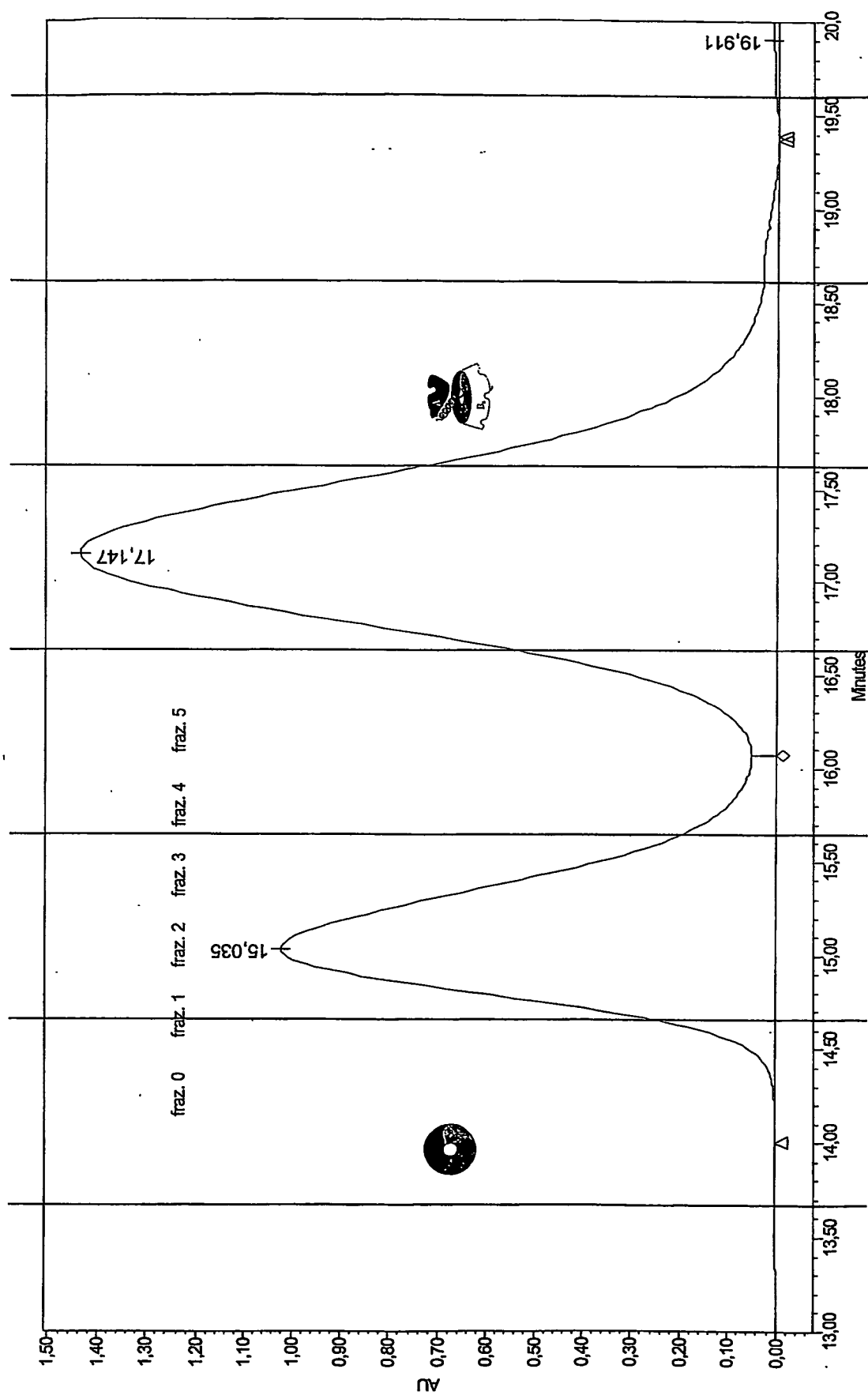


Figure 4



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Figure 5A

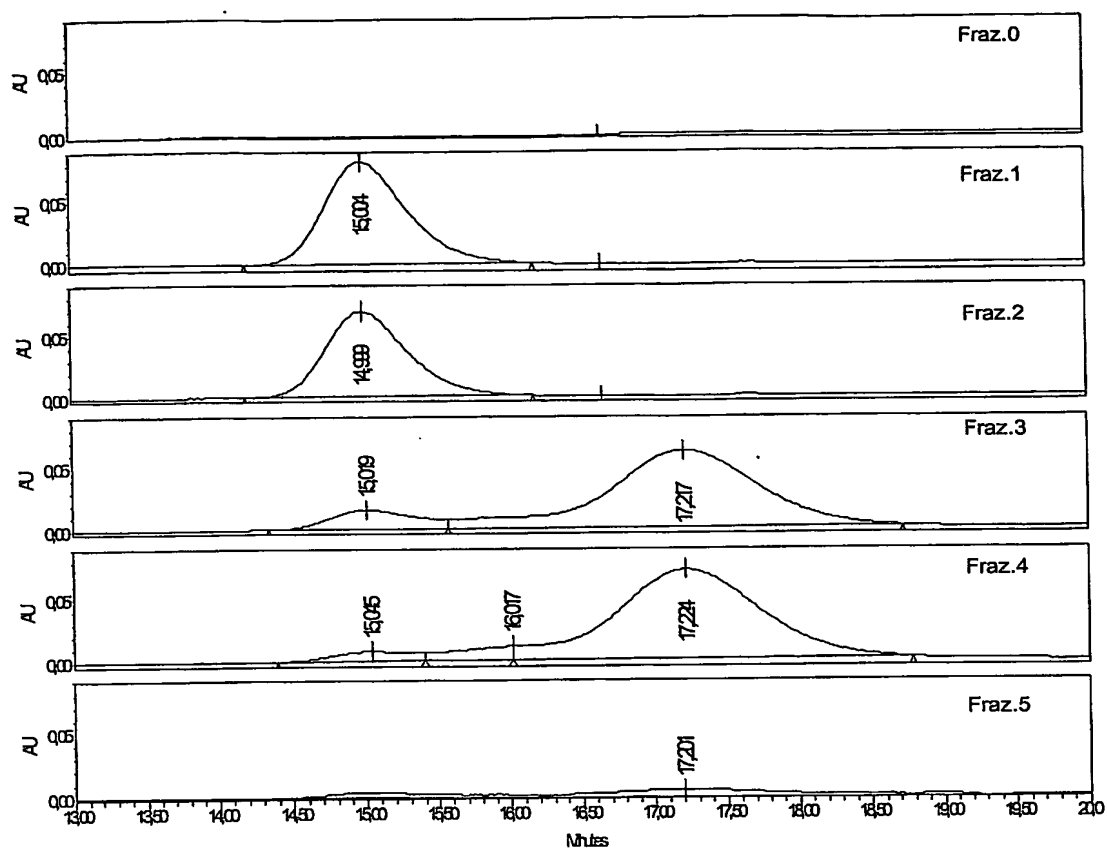


Figure 5B

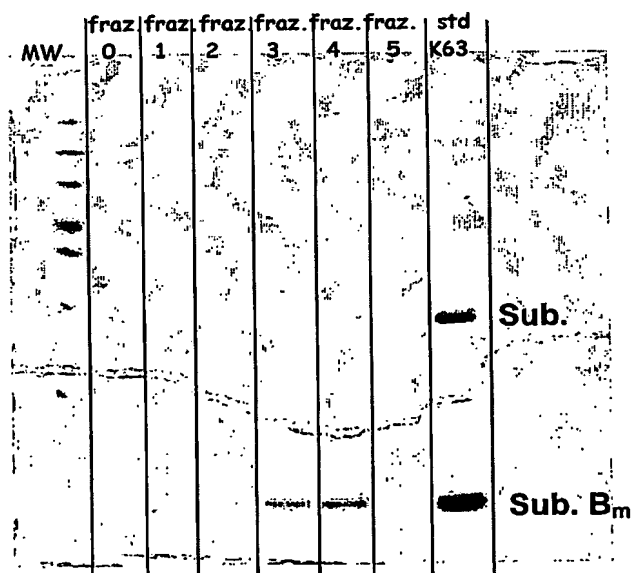
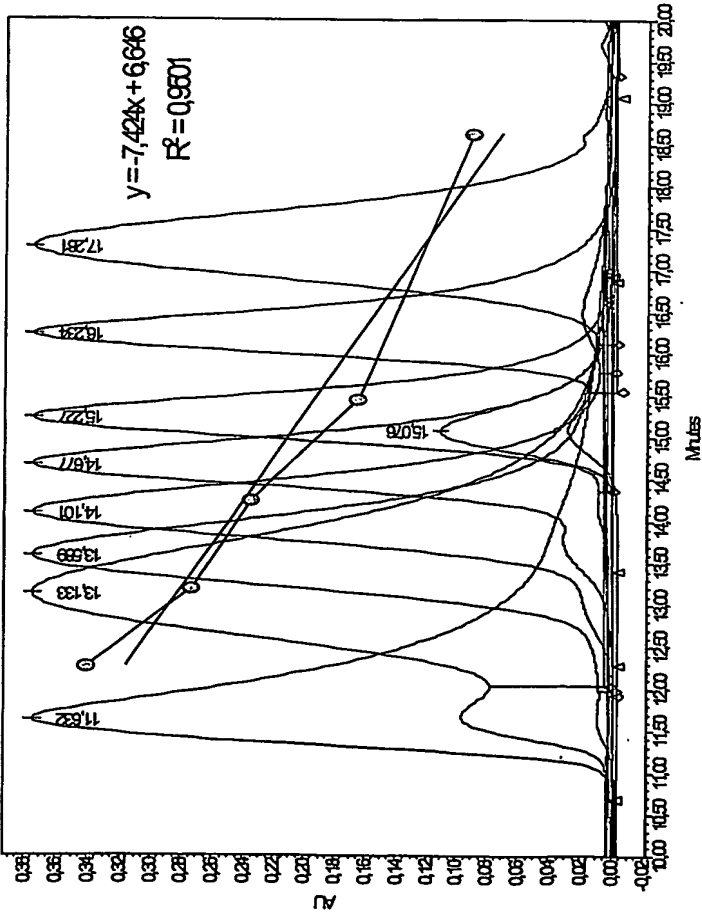


Figure 5D

Standard proteins	Rt (min)	M _w (Da)
Thyroglobulin (bovine)	11.62	669.000
Apoferitin	13.13	476.316
B-amylase	13.58	224.340
Alcohol Deydrogenase	14.10	146.980
BSA	14.67	66.800
Carbonic Anhydrase	16.22	29.023
Sample proteins	Rt (min)	M _w exp.
CRM	15.23	57.099
K63 AB ₅	17.26	9.611
K63 B ₅	15.07	65.607

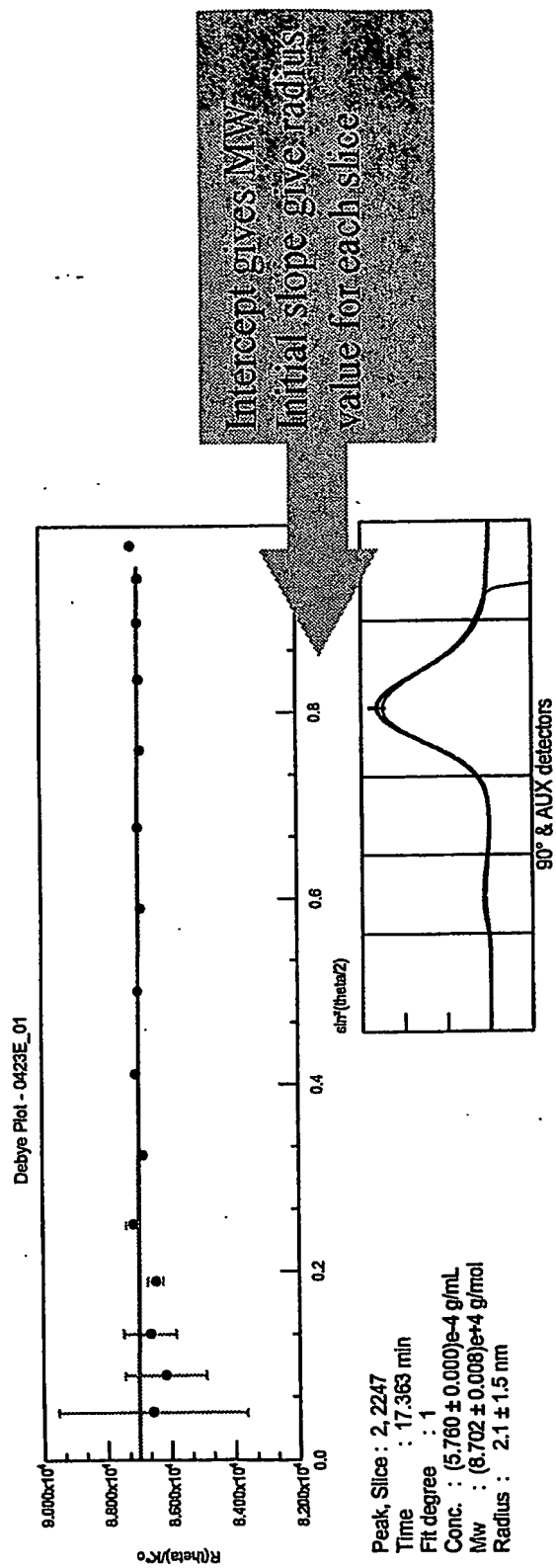
Figure 5C



Superimposition of standard proteins, CRM197, reference (bold blue), K63 (bold red) and calibration curve used for apparent MW determination.

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Figure 5E



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Figure 5F (a)

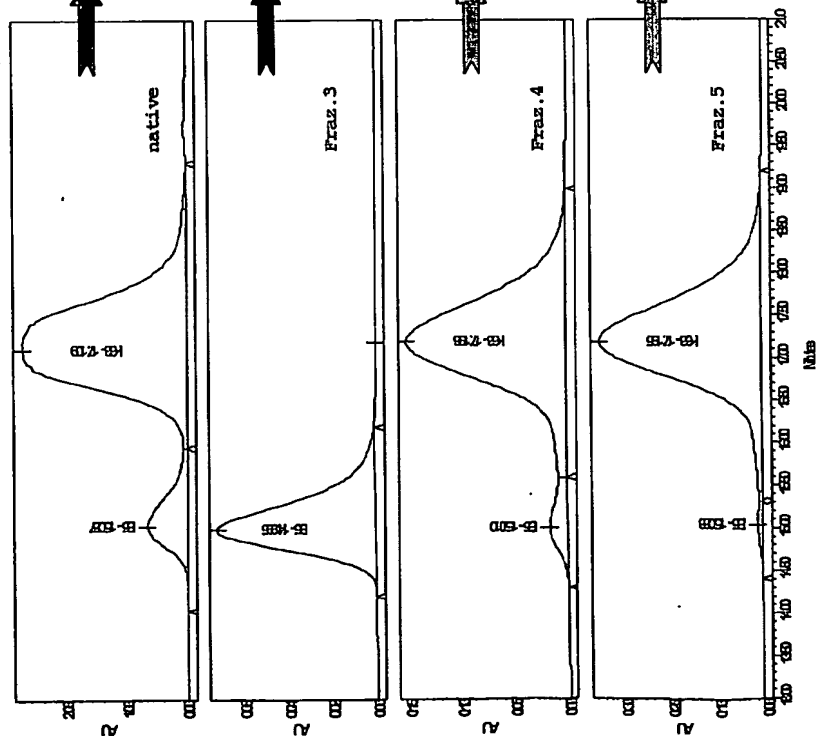
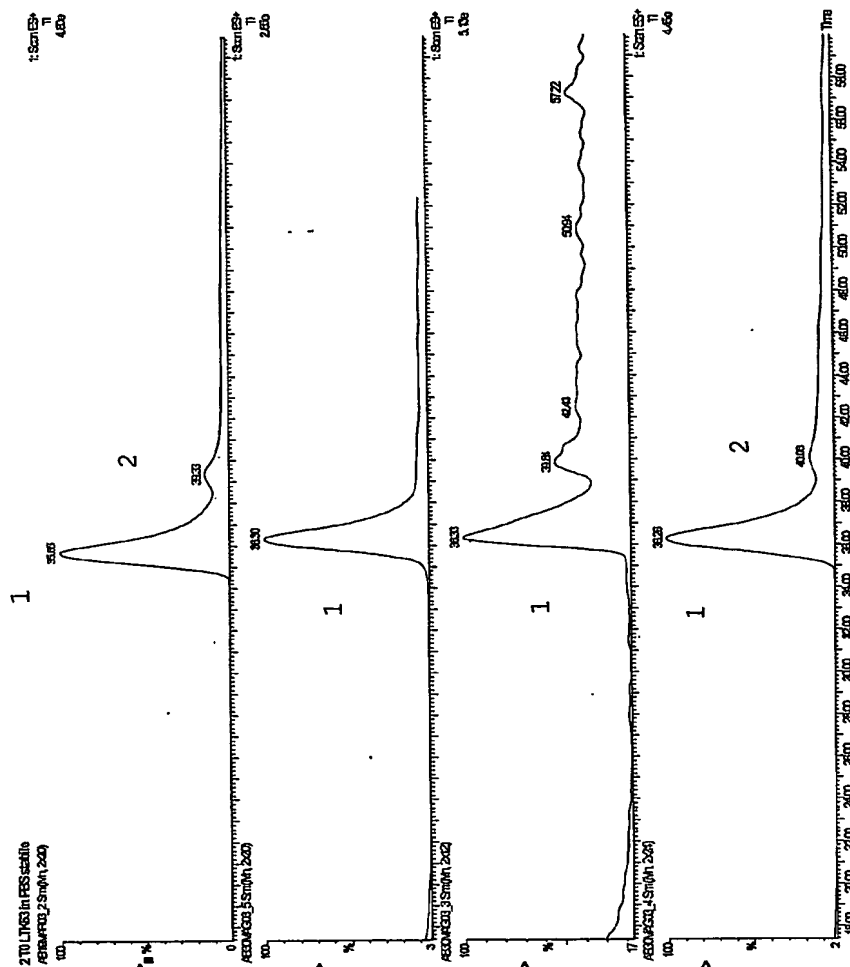
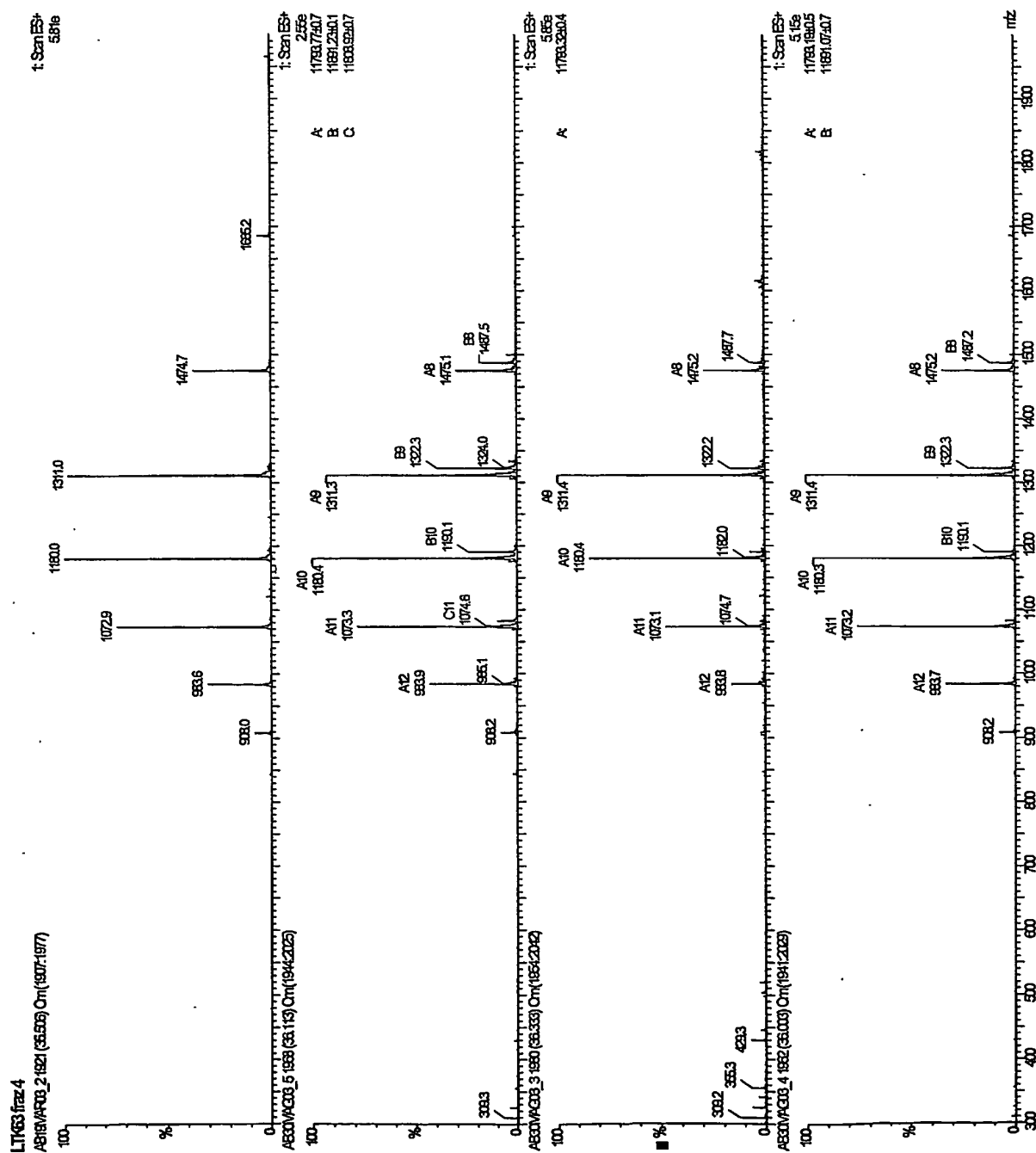


Figure 5F(b)



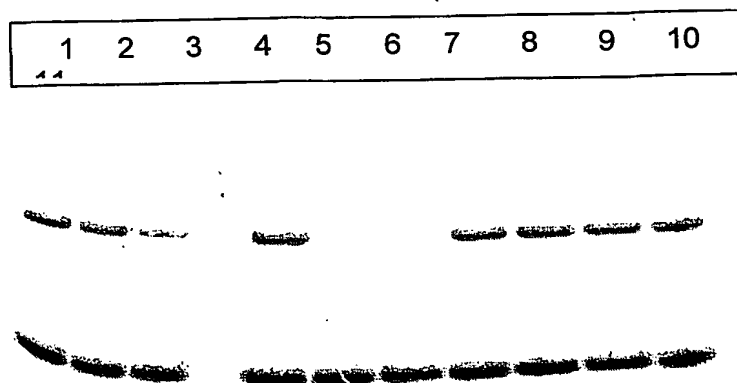
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Figure 5G



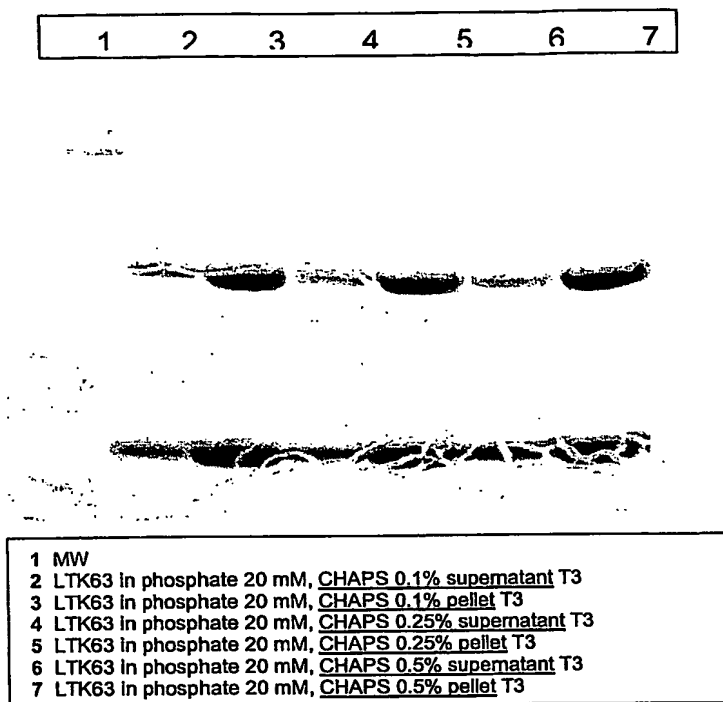
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Figure 6: SDS-PAGE analysis of LTK 63 shaken samples



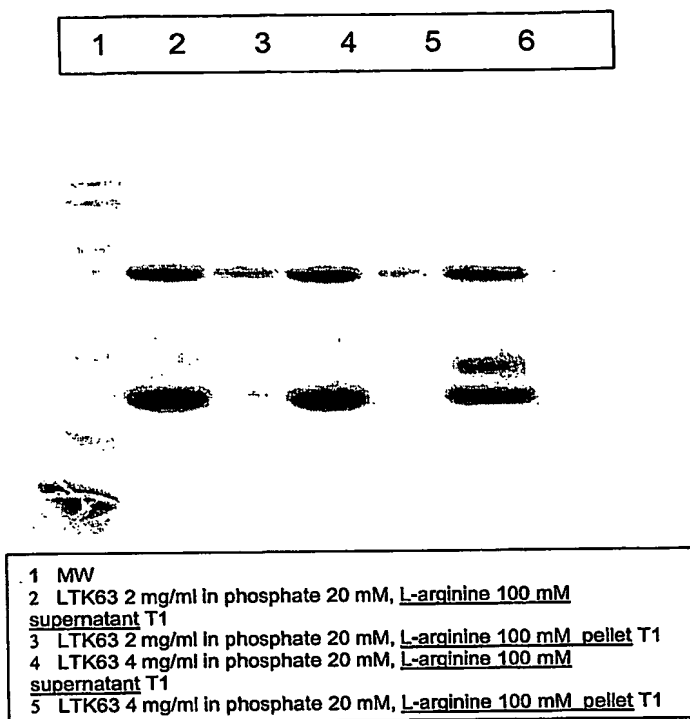
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Figure 7: SDS-PAGE analysis of LTK 63 samples treated with CHAPS

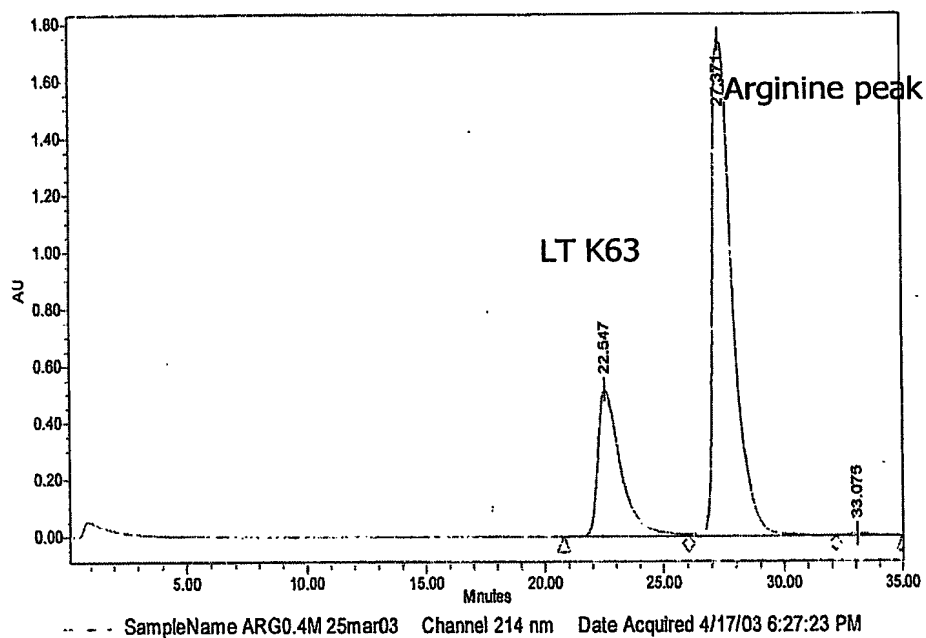
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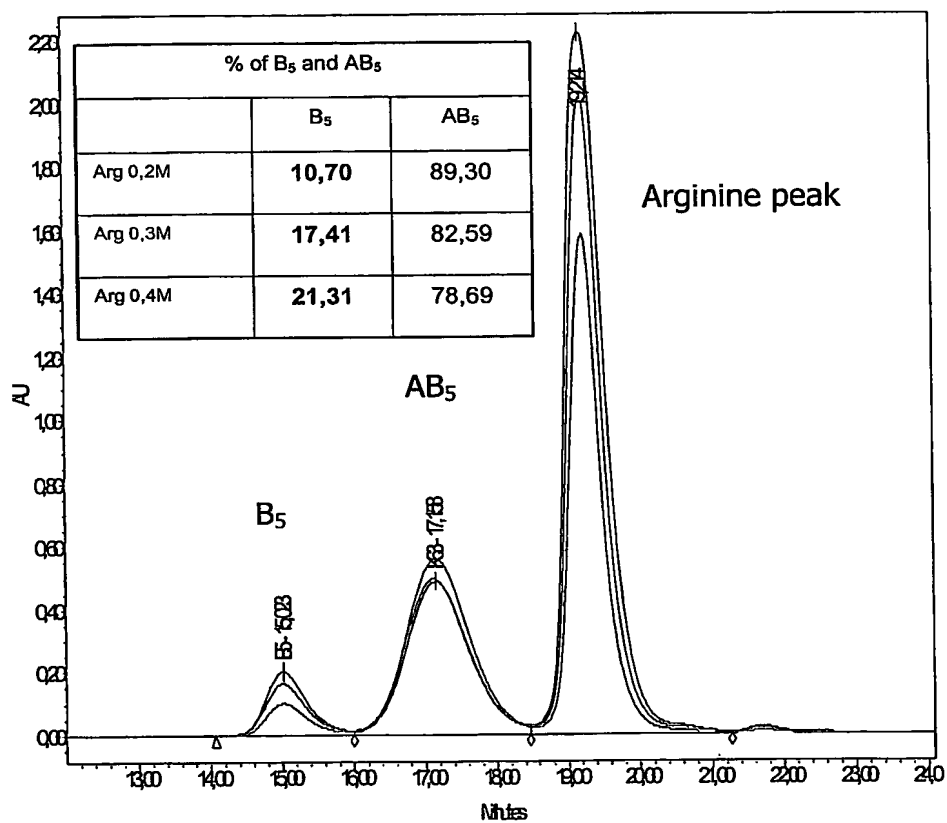
Figure 8: SDS-PAGE of LTK63 samples treated with L-Arginine

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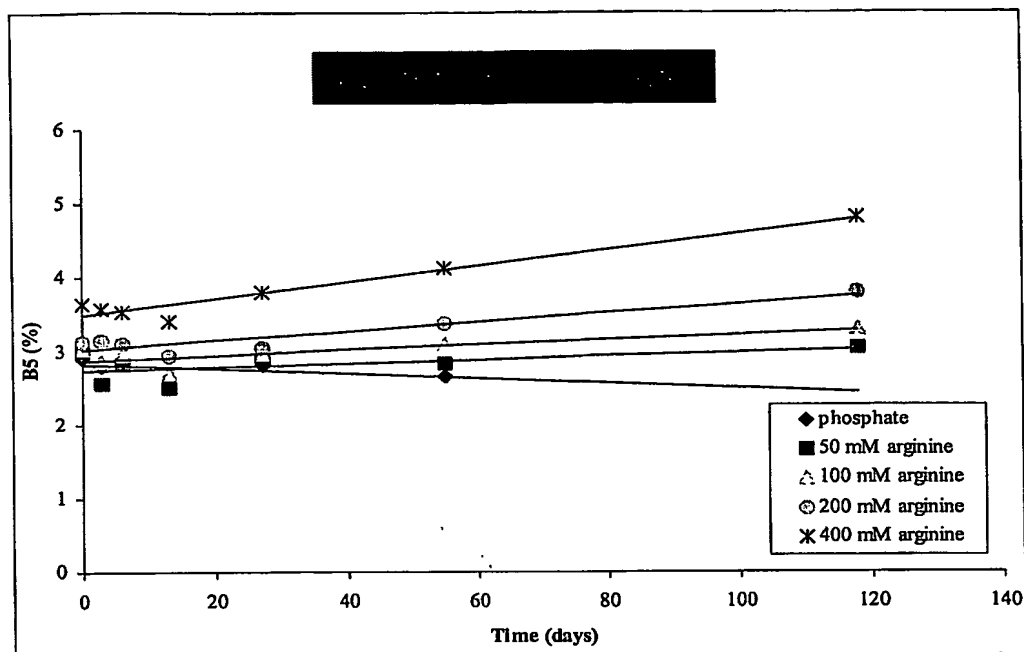
Figure 9(a): Old HPLC Method for analysing L-Arginine treated samples

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Figure 9(b): New HPLC Method for analysing L-Arginine treated samples

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Figure 10(a): Determination of AB5 dissociation in L-Arginine treated samples and the %B5 in LTK63 at 1.3mg/ml



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Figure 10(b): Determination of AB5 dissociation in L-Arginine treated samples and the %B5 in LTK63 at 4.0mg/ml

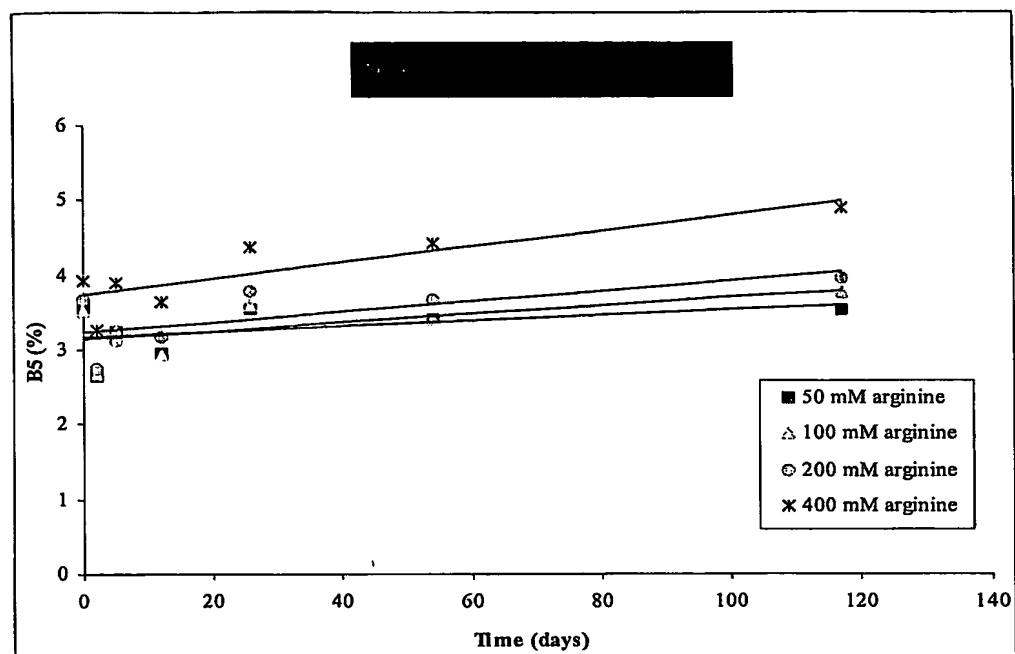
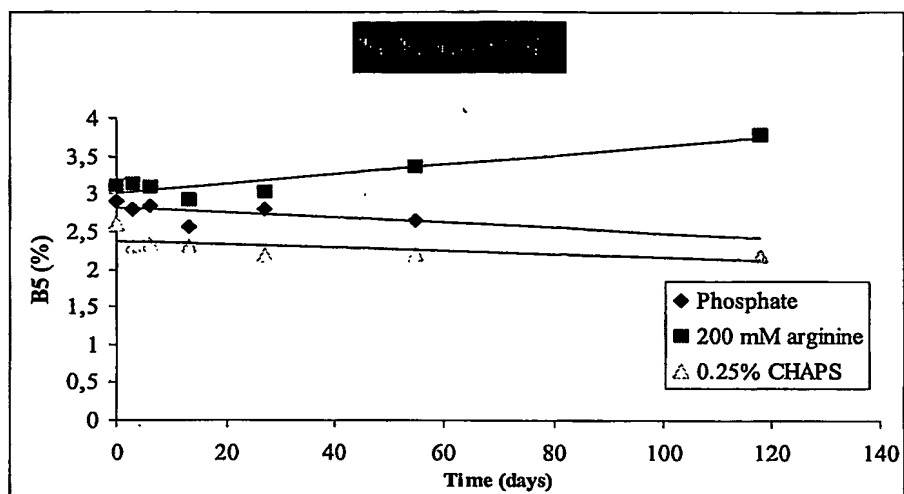


Figure 11(a): CHAPS effect on LTK63 dissociation

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Figure 11(b): CHAPS effect on LT K63 dissociation in combination with L-Arginine

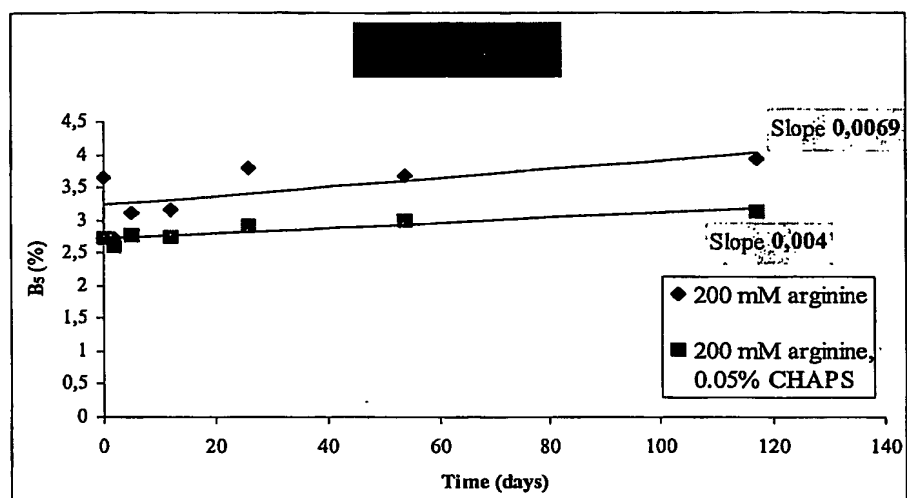


Figure 12: Effect of L-Arginine and CHAPS on LTK 63 stability at a protein concentration of 1,3 mg/ml

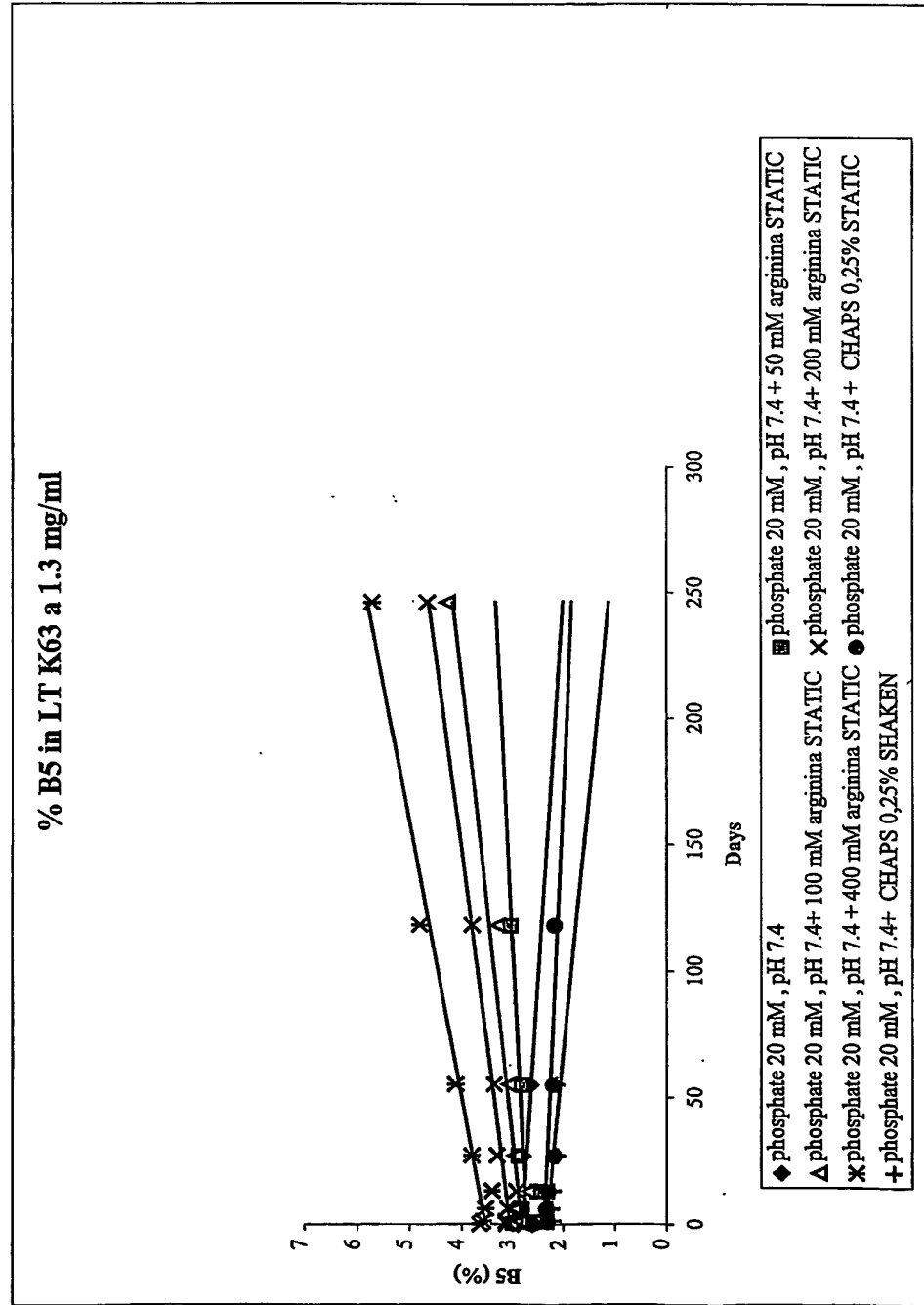
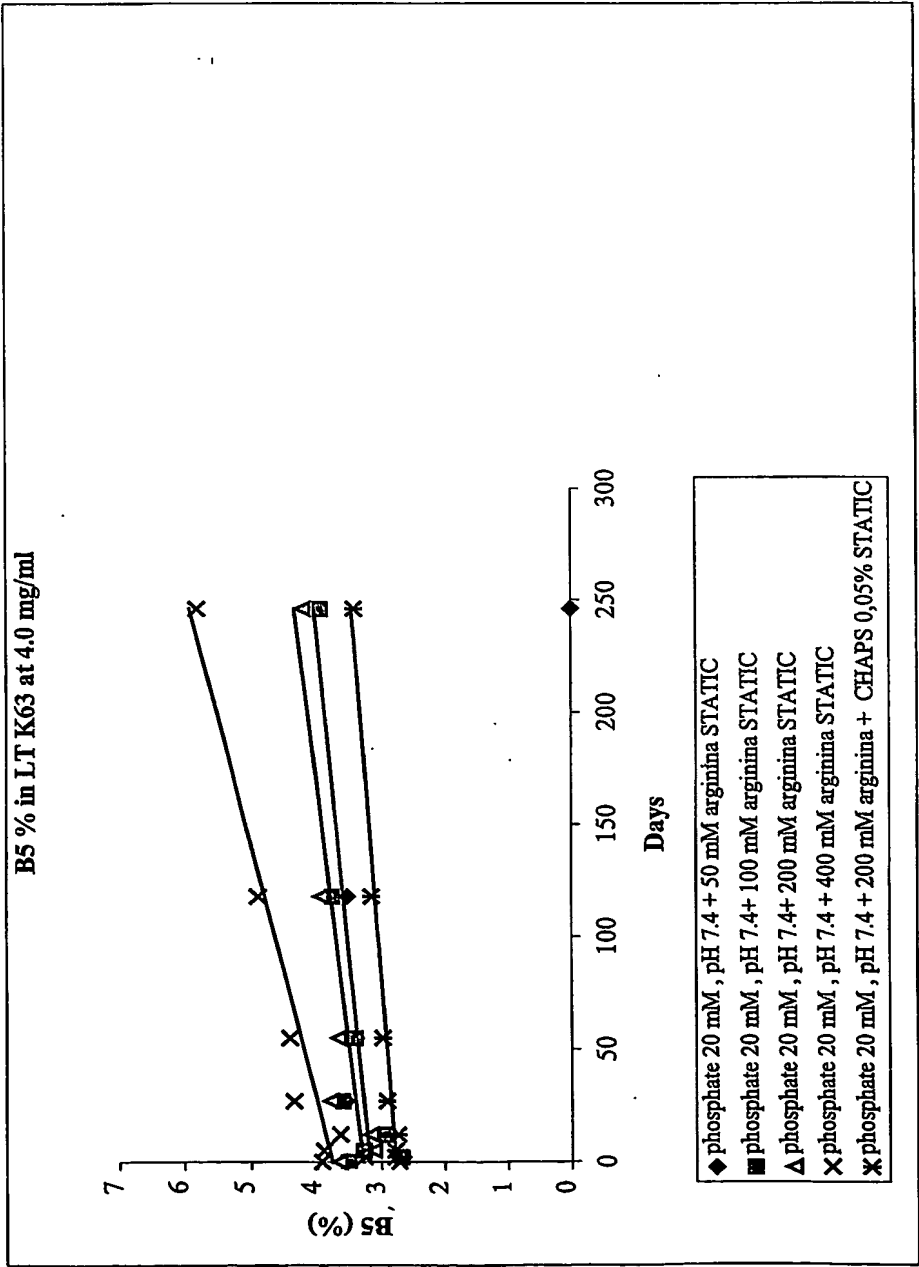


Figure 13: The effect of L-Arginine and the combination L-Arginine/CHAPS on LTK 63 stability at a protein concentration of 4,0 mg/ml



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Figure 14 shows the effect of storage conditions on LTK 63 stability in L-Arginine + CHAPS containing buffers

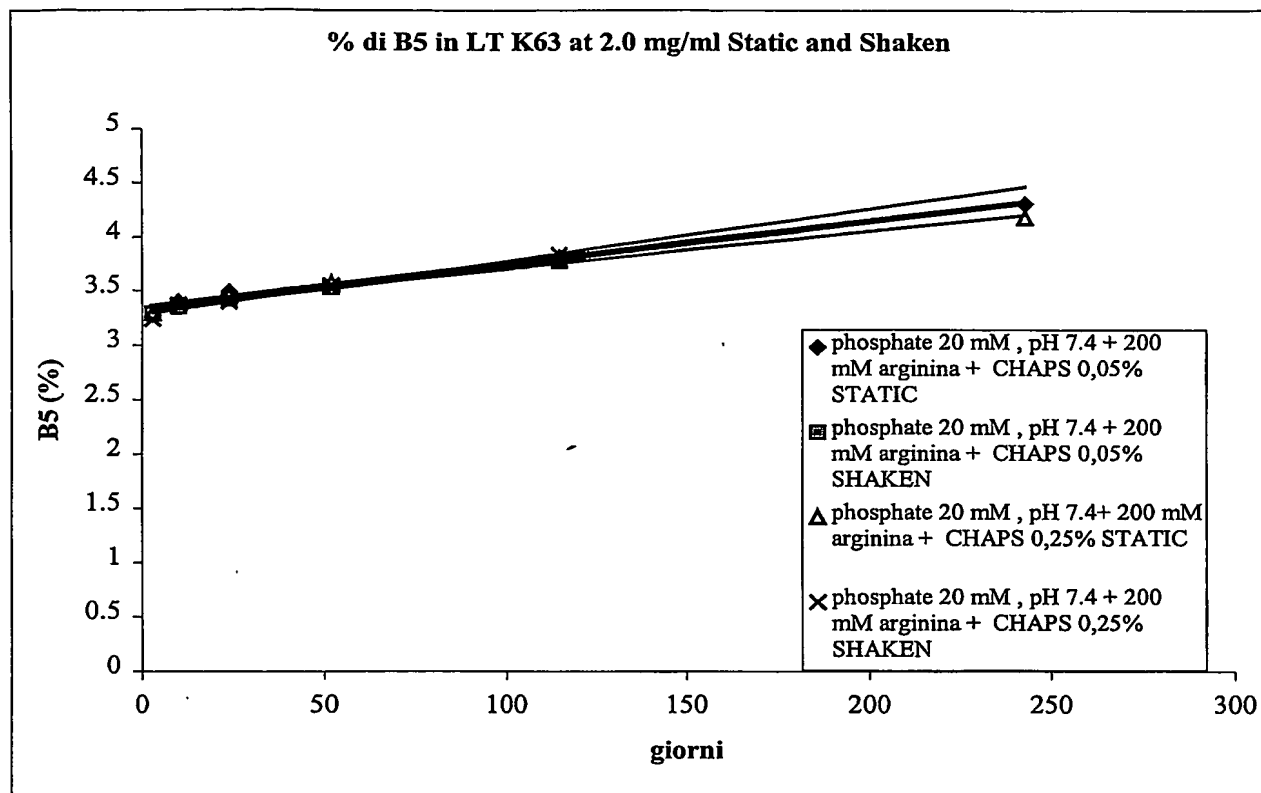


Figure 15: Comparison of LTK 63 stability on L-Arginine and L-Arginine + CHAPS storage buffers

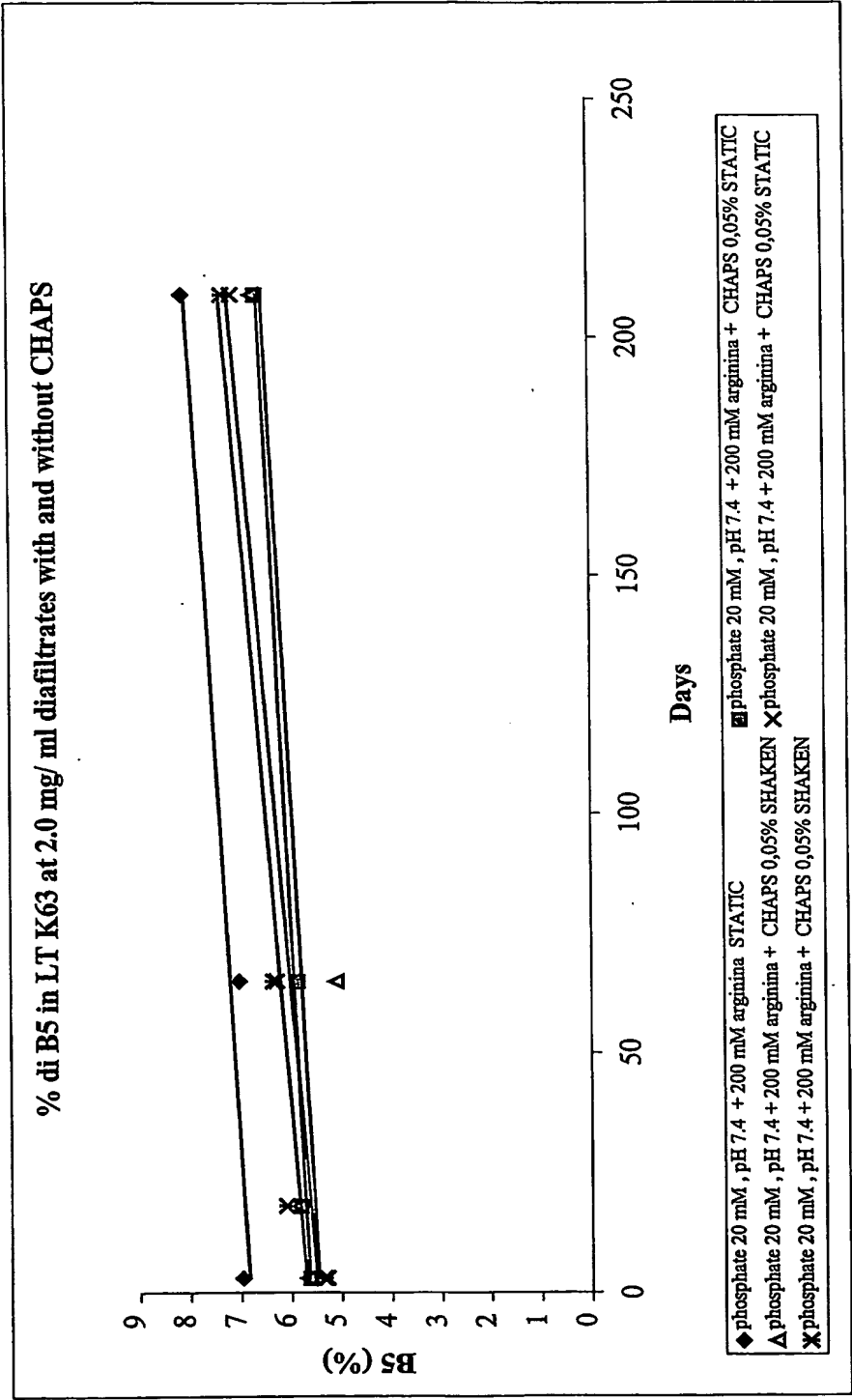


Figure 16

Table 2. Structure and Classification of Detergents (continued)	
<p data-bbox="868 1753 901 1890">Zwittergents</p> <div data-bbox="438 966 568 1449"> $\text{CH}_3(\text{CH}_2)_{11}-\text{N}^+(\text{CH}_3)_2-\text{CH}_2-\text{COO}^- \quad \text{pH} \geq 6$ </div> <div data-bbox="714 1092 836 1449"> $\text{CH}_3(\text{CH}_2)_x-\text{N}^+(\text{CH}_3)_2-(\text{CH}_2)_3-\text{SO}_3^-$ </div> <div data-bbox="1023 777 1299 1533"> </div>	<p data-bbox="381 336 462 682">EMPIGEN BB® (n-dodecyl)-N,N-dimethylglycine</p> <p data-bbox="706 336 893 682"> x = 7, ZWITTERGENT® 3-08 x = 9, ZWITTERGENT® 3-10 x = 11, ZWITTERGENT® 3-12 x = 13, ZWITTERGENT® 3-14 x = 15, ZWITTERGENT® 3-16 </p> <p data-bbox="1055 483 1128 682"> x = H, CHAPS x = OH, CHAPSO </p>

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Figure 17

Zwitterionic Detergents

Product	Cat. No.	M.W. (anhydrous)	CMC ^b (mM)	Aggregation No.	Average Micellar Weight	size
ASB-14	182750	434.7	—	—	—	5g, 25 g
ASB-16	182755	462.7	—	—	—	5g, 25 g
CHAPS	220201	614.9	6-10	4-14	6000	1 g 5 g 10 g 25 g
CHAPSO	220202	630.9	8	11	7000	1 g 5 g
DDMAB	252000	299.5	4.3	—	—	5 g
DDMAUS	252005	319.7	0.1	—	—	5 g
EMPIGEN BB [®] Detergent, 30% Solution	324690	272.0	1.6-2.1	—	—	100 ml
Lauryldimethylamine Oxide (LDAO) 50% Solution	493011	279.4	1.2	76	17,000	100 ml
ZWITTERGENT [®] 3-08 Detergent	693019	279.6	330	—	—	5 g
ZWITTERGENT [®] 3-10 Detergent	693021	307.6	25-40	14	12,500	5 g 25 g
ZWITTERGENT [®] 3-12 Detergent	693015	335.6	2-4	55	18,500	5 g 25 g
ZWITTERGENT [®] 3-14 Detergent	693017	363.6	0.1-0.4	183	30,000	5 g 25 g 100 g
ZWITTERGENT [®] 3-16 Detergent	693023	391.6	0.01-0.06	155	60,000	5 g 25 g

a. Average molecular weights are given for detergents composed of mixtures of chain lengths; b. Temperature: 20 - 25°C

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